The Potential of Ireland's Native Three-Spined Stickleback (*Gasterostues aculeatus*) for the Biological Control of Mosquito Larvae (Subfamily: *Culicinae)* in Ballyvergan Marsh, Youghal, Co. Cork.

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CARL Research Project

in collaboration with

Youghal Tidy Towns

COMMUNITY - ACADEMIC RESEARCH LINKS

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- provide their services on an affordable basis;
- promote and support public access to and influence on science and technology;
- create equitable and supportive partnerships with civil society organisations;
- enhance understanding among policymakers and education and research institutions of the research and education needs of civil society, and
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ABSTRACT

Marshes provide suitable habitats for larval development of nuisance and vector mosquitoes worldwide. Ecological and ecotoxicological consequences of traditional methods have forced mosquito management to less destructive approaches such as Open Marsh Water Management (OMWM); a technique that promotes larval control by tidal flushing and giving native predatory fish access to isolated larval habitats. However, management schemes such as OMWM are rare in European marshes and non-existent in Ireland. Ballyvergan marsh is a coastal marsh located on the south-east coast of Ireland that supports populations of three-spined stickleback, *Gasterosteus aculeatus,* within a tidal creek and *Aedes* and *Culex* mosquito larvae (Subfamily: *Culicinae*) in isolated, brackish pools. A field study was conducted to determine the biological control potential of three-spined sticklebacks against mosquito larvae by investigating 1) the predator-prey interactions between three-spined sticklebacks and *Culicinae* larvae in three different sub-habitats of the marsh 2) the functional response of three-spined sticklebacks in brackish and freshwater 3) the consumption rates as a function of group size. All experiments were conducted in controlled *in situ* conditions using 10L plastic buckets with mesh windows. Sticklebacks showed strong biological control potential, consuming larvae across different sub-habitats of the marsh. A Type II functional response in brackish and freshwater was identified with an estimated maximum consumption rate of 429 ± 32 larvae per pair of sticklebacks in 24 hours. It is suggested that management methods, such as OMWM would control local mosquito populations in Ballyvergan marsh through predation by three-spined sticklebacks. There is an increasing emphasis on the need to apply ecologically sound mosquito control solutions, as the risk of re-emerging vector-borne diseases in Europe continues to rise with climate change.

INTRODUCTION

There are over 3,500 species of mosquitoes in the world (Wang et al., 2012), approximately 100 of which are vectors for human diseases such as malaria, chikungunya, dengue, zika virus disease and West Nile fever (WHO, 2020a) Mosquito-borne diseases infect up to 700 million people across 100 countries, with approximately 2.7 million fatal cases each year (Ghosh et al., 2012; Rozendaal, 1997). While the majority of vector mosquito populations are established in tropical and subtropical regions (WHO, 2020b), the risk of locally transmitted outbreaks of mosquito-borne diseases are becoming more progressive in continental Europe, particularly in the Mediterranean (Papa, 2019). Invasive species *Aedes aegypti* and *Aedes albopictus* are an ongoing threat to continental Europe, bringing increased risk of dengue and chikungunya outbreaks (Akiner et al., 2016). Invasive *Aedes* mosquitoes are usually introduced anthropogenically via the travel and trade industry, and if conditions are right, the species can establish local populations (Akiner et al., 2016). Multiple factors such as high ecological and physiological plasticity, land use change, increased urbanisation and climatic factors such as temperatures and precipitation all contribute to the successful colonisation of invasive vectors (Brugueras et al., 2020; Wilke et al., 2020). Predictions of a future wetter and warmer climate between 2030-2050 will potentially bring an increased risk of colonisation of *Aedes albopictus* in central western Europe, including the UK (Caminade et al., 2012). The effects of climate change also increase the risk of disease carried by native mosquito populations. Warmer air temperatures are correlated to outbreaks of West Nile fever in higher latitudes, where endemic outbreaks do not normally occur (Paz et al., 2013). Within the last two years, human cases of locally transmitted West Nile virus have emerged in north-east Germany from native populations of *Culex* mosquitoes as a result of viral overwintering (Holicki et al., 2020; Kampen et al., 2020).

While malaria, a disease caused by protozoic parasites has not made it past the tropics and subtropics in recent years, evidence suggests that *Plasmodium vivax* malaria, was once endemic in the UK from the 14th century until the early 20th century. Although it was not known to be malaria at the time, the disease was termed as a tertian or quartan ague denoting the intermittent fevers that occurred every third or fourth day. These fevers are now known as diagnostic symptoms of malaria. In the 17th and 18th century, some reports of ague were associated with hot summers in marshy areas (Chin and Welsby, 2004; Hutchinson and Lindsay, 2006). Malaria in the UK began to decline from the 19th century, likely as a result of marsh drainage, improved standard of living and the availability of antimalarial drugs such as quinine. Increased travel and trade paired with climate change means that the chances of re-emerging indigenous malaria in the next few decades is not unlikely (Chin and Welsby, 2004). While marsh mosquitoes do not pose serious human health risks at current, mosquitoes in coastland areas are often considered nuisance species that can reduce quality of life as well as result in local economic loss in relation to tourism and agriculture. In these cases, control of nuisance species in populated areas should be considered (Cheng, 1976; Rey et al., 2012). Because mosquitoes are dependent on an aquatic larval stage, pools and inundated depressions in marshes provide suitable habitats for the oviposition of coastal mosquitoes. Controlling mosquitoes by targeting the larval stages are highly effective if habitats are large and are easily modified (CDC, 2020). In addition, larvae found in marshes occur in high densities and in predictable habitats, making larval control the rational management method over adult control. The practice of intensive larval control can be dated back to the early 20th century, where it was particularly prevalent along the Atlantic Coast of North America (Clarke et al., 1984). Early control methods often involved source reduction, chemicals and the use of larvivorous fish.

Source Reduction: Source reduction methods involve physical manipulation of marsh in order to impede oviposition. Mosquito impoundment describes the process in which a dike is constructed around the perimeter of the marsh so that the area can be temporarily flooded during the breeding season (Rey and Connelly, 2001). Grid-ditching involves the formation of parallel drainage ditches 50-100m apart across the entire marsh in order to drain pools and depressions that provide suitable habitats for oviposition and larval development. Grid-ditching was the prevalent form of source reduction control in the early 20th century in North America and by 1930, 90% of saltmarshes along the Atlantic coast had been ditched. However, both management methods often have many ecological consequences. Impoundment can change species composition and prevents access of estuarine fish (Montague et al., 1987). Grid ditching reduces access to food and habitat use of coastal birds (Clarke et al., 1984) while also affecting hydrology, surface elevations and soils (Vincent et al., 2012).

Biological Control: Biological control of mosquitoes aims to keep populations to an 'acceptable' level that protects humans, conserves biodiversity and does not cause any eco-toxicological effects (Becker et al., 2010). Fish are the biggest predators of mosquito larvae in saltmarshes and therefore have been used as suitable biological control agents (Cheng, 1976). The western mosquitofish *Gambusia affinis* and the eastern mosquitofish *Gambusia holbrooki*, both native to the United States, are the most widely used larvivorous fish in mosquito biocontrol since the early 20th century (Kumar and Hwang, 2006). However, they have become globally invasive from regions in the United States (Laha and Mattingly, 2007; Leyse and Lawler, 2004), to the Mediterranean in Europe (Cabrera-Guzmán et al., 2017; Rincón et al., 2002) and to Australia (Kerezsy and Fensham, 2013), threatening biodiversity loss of native fishes and amphibians. In an effort to avoid such detrimental effects, the use of native larvivorous fish as an alternative control method has led to some successful outcomes (Bonifacio et al., 2019; Chandra et al., 2008).

Modern control practices: Although early mosquito control methods resulted in the ecological destruction of marshes, the foundations of these methods are still in use today. Open Marsh Water Management (OMWM) is a term that refers to the physical manipulation of marsh habitat to make it less amenable to mosquito populations. OMWM has been a successful, less destructive method of controlling mosquitoes along the Atlantic coast of North America since the 1960's and combines source reduction and biological control. OMWM systematically creates permanent pools and radial ditches within the marsh habitat to reduce mosquito populations by enhancing tidal circulation among oviposition/larval sites and providing access to local predatory fish, thus facilitating biocontrol. It is far less destructive than traditional grid ditching and restores surface water levels (Wolfe, 1996). A simpler management design called 'runnelling', is a common method used in Australia in which wide, shallow ditches are dug to connect small mosquito breeding depressions to a tidal water source. (Dale et al., 1993, 1989; Dale and Knight, 2006). The key aim of both methods is to connect isolated pools that support mosquito larvae but are not accessible to predatory fish, to fluvial or tidal waters via artificially dug channels, thus allowing fishes to colonise pools and consume larvae. Application of OMWM and 'runnelling' are well established and documented in the United States and Australia. However, resources for the management of salt marsh mosquitoes in continental Europe and the British Isles are scarce (Medlock and Vaux, 2011) and there is currently no evidence of mosquito control practice in Irish marshes. While control of mosquitoes in Ireland may not seem like an urgent issue, increased nuisance levels and risk of diseasing bearing mosquitoes in the future is likely as a direct and indirect result of changing climatic conditions (Blagrove et al., 2016; Medlock and Vaux, 2011).

The three-spined stickleback, *Gasterosteus aculeatus*, is a small, teleost fish found on every continent across the northern hemisphere in saline, brackish and freshwaters (Bell and Foster, 1994; Wootton, 1976). They are generally categorised into anadromous or resident freshwater types. However, sticklebacks in intertidal zones such as salt marshes are often resident species and do not migrate into freshwaters (Arai et al., 2020). Due to their extensive geographical and habitat ranges, sticklebacks show a high degree of variation in morphological, physiological and behavioural traits between populations (Bell and Foster, 1994; Mccairns and Bernatchez, 2012; Rind et al., 2020). Stickleback morphotypes are determined by local adaptations in response to abiotic and biotic conditions. Food availability is just one of many selective pressures that causes distinct morphotypes through local adaptation (Bell and Foster, 1994; Willacker et al., 2010). As a result, diet and feeding behaviour of different stickleback morphs lie along a benthic-limnetic continuum, feeding from a wide variety of benthic macroinvertebrates to pelagic zooplankton (Baker et al., 2008; Willacker et al., 2010). Three-spined sticklebacks are present in Irish saltmarshes year-round, becoming more common during the months of May-November with the highest abundance during the summer months following the breeding season (Koutsogiannopoulou and Wilson, 2007; O'Sullivan, 1984, 1983). However, resources on the biology and ecology of Irish sticklebacks such as their feeding behaviour and diet is limited. Information on the use of sticklebacks for biological control of mosquitoes is also limited, despite that they have been reported to consume mosquito larvae when offered as a prey item (Medlock and Snow, 2008). Furthermore, sticklebacks demonstrate some similar characteristics of mosquitofish (*Gambusia spp.*) that make them successful biocontrol agents, including a high tolerance to hypoxic conditions and organic pollution (Katsiadaki et al., 2007; Regan et al., 2017; Stoffels et al., 2017). Other characteristics that sticklebacks share with general larvivorous fish include an upturned mouth and teeth, a small body size that is fusiform in shape, tolerance to stressful environmental conditions and they are highly agile in shallow water (WHO, 2003).

This study investigates the control potential of Ireland's native three-spined stickleback *Gasterosteus aculeatus* on *Culicinae* mosquito larvae under controlled conditions in Ballyvergan Marsh located in Youghal, Co. Cork. We quantified the predator-prey interactions and the biological control potential between stickleback and mosquito populations by determining:

1) whether sticklebacks will consume mosquito larvae in different sub-habitats of the marsh
2) the consumption rate of sticklebacks as a function of prey density (functional response) in fresh and brackish water
3) the consumption rate of sticklebacks as a function of stickleback group size

METHODS AND MATERIALS

Site Description

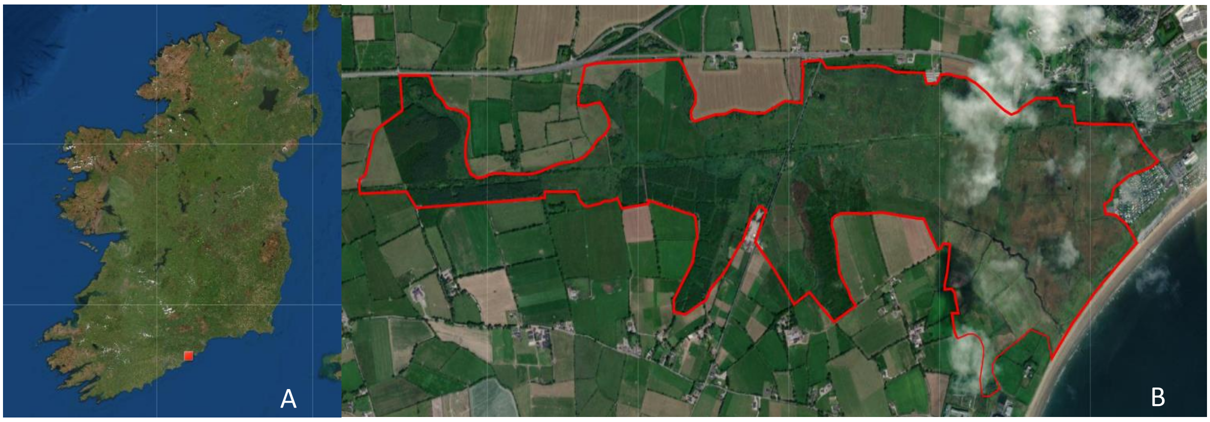

Fig. 1 Satellite imagery of the general location of Ballyvergan marsh on a national scale (A) and the pNHA boundary of Ballyvergan marsh (B).

Ballyvergan marsh is Irelands largest freshwater coastal marsh (158 ha) located in Youghal, Co. Cork (Goodwillie, 1986) and is listed as a proposed Natural Heritage Area (pNHA) under the National Parks and Wildlife Services for its significant value as a wildlife habitat (fig. 1). An ecological assessment report conducted by Wild Work, a SECAD initiative, in 2017 identified increased saline influences in the south-east end of the marsh. A compromised sluice gate that is no longer in operation rapidly fills a tidal creek during each high tide, filling old drainage ditches that now form secondary channels, leading to tidal pools (fig. 2). The study area was located in the south-east end of the marsh that is subject to saline influence from the tidal creek (fig. 2).

Fig. 2 Satellite image of the study area in the south-east end of Ballyvergan marsh. The tidal, isolated and permanent pools represent the study sites.

Shallow pools and depressions of stagnant, brackish water throughout the marsh provided suitable habitats for *Culicinae* larvae (predominantly *Aedes,* but *Culex* were also identified). These pools were isolated from any tidal flushing and were not connected by the tidal creek, a habitat that supports shoals of three-spined stickleback.

Observations and salinity measurements during several walkovers of the marsh in August and September 2020, identified three distinct pool types (fig. 2). The first was a permanent shallow pool sheltered by common reed, *Phragmites australis*, in the lower marsh. This pool had the lowest salinity of 10.9 mS/cm suggesting that it is supplied by a freshwater source. However, it was still relatively brackish and had a secondary channel connected to the tidal creek indicating some saline influence. Three-spined sticklebacks were caught and identified in the permanent pool by using a hand-held net and sweeping along the vegetation. Further into the marsh, a tidal pool was identified, characterised by a lack of tall, sheltering vegetation both within and surrounding the pool. It was located in close proximity to the tidal creek and had a higher salinity measurement of 32.4 mS/cm, which matched that of the creek. Sweep samples did not show any evidence for the presence of three-spined sticklebacks or mosquito larvae. Scattered around the marsh, a third pool type was identified. These pools were generally smaller in size, completely cut off from the tidal creek or any secondary channels and dominated by sedges and/or floating algae. Samples found mosquito larvae, but no evidence of colonisation by three-spined sticklebacks. A summary of the observations and findings is presented in Table 1.

Table 1. Summary of the descriptive observations of the three pool types, including salinity (mS/cm) and presence of sticklebacks and/or mosquito larvae during the site walkover

Pool	Description	Salinity (mS/cm)	Sticklebacks	Mosquitoes
Permanent	Large, shallow pool, sheltered by reed bed. Furthest distance from tidal creek	10.0	Yes	No
Tidal	Large, shallow pools, highly exposed. Close proximity to the tidal creek, connected via secondary channels	32.4	No	No
Isolated	Cut off from any tidal channels. Dominated by dry/dead sedges and/or floating algae	14.0	No	Yes

Materials

To quantify the predator-prey interactions and investigate the biological control potential of three-spined sticklebacks against mosquito larvae, the study consisted of three controlled *in-situ* experiments. The experiments were completed in 10 litre plastic buckets with 8 x 16cm cut-out windows that were covered using net curtain fabric with a 1mm sized mesh. The fabric was secured to the cut-out with a certified fish-safe waterproof glue. This allowed the buckets to keep both fish and larvae in a contained environment during the study, while the mesh windows permitted water exchange to keep abiotic conditions homogenous with the surrounding pool.

Procedure for collecting sticklebacks and mosquito larvae

Sticklebacks were collected from the tidal creek by standing on the bank and sweeping under the overhanging vegetation using a hand-held net and transported in a closed container with water from the tidal creek to each treatment site. Before being added to buckets at a treatment site, the required number of sticklebacks were removed and placed in a separate container of water from the tidal creek. They were then acclimated to the given pool by gradually adding water every few minutes from the treatment site into the container. The sticklebacks were released back into the tidal creek after each experiment was carried out. For each experiment, a large sample of larvae of various instar were collected from a selected, isolated pool by dipping a small plastic container under the surface. The larvae were filtered out using a fine mesh aquarium net and placed into a bucket of water for transportation to each treatment site.

Experimental Design and Methodology

1. Predator-prey interactions under three pool types

Predator-prey interactions were quantified under three treatments; the permanent pool, the tidal pool and the isolated pool, identified during the walkovers. Due to an extended dry period from August-September, many areas of the marsh had temporarily dried out, leaving insufficient accessible isolated pools for replication. As a result, only one pool of each type was used in the experiment. Experimental buckets were randomly placed in pairs (one as the test bucket, one as the control bucket) and pseudo-replicated three times within each pool (fig. 3) A large stone was placed in the bottom of each bucket to secure it in place. Twenty larvae were added to both test and control buckets, followed by a pair of sticklebacks in each test bucket. After a 48-hour trial period, the remaining larvae in the test and control buckets at each treatment site were counted. This process was repeated two more times to achieve a total of three trials.

Fig. 3 Experimental set-up of the predator-prey interactions between three-spined sticklebacks and *Culicinae* larvae in the permanent (A), tidal (B) and isolated (C) pools in Ballyvergan

2. Consumption rates as a function of prey density in freshwater vs brackish water

The efficiency of three-spined sticklebacks as a biological control agent was quantified by establishing their functional response under two treatments; fresh water and brackish water in a secluded part of the marsh. Each treatment had seven buckets of increasing larval densities {50, 100, 200, 300, 400, 600, 1000 individuals per bucket}. As counting was a time-consuming process, the trial with the largest density of 1000 individuals/bucket was run separately. Pairs of previously collected sticklebacks were randomly selected, acclimated and placed into all buckets for 24 hours. After the trial period, sticklebacks were removed and remaining larvae were counted and recorded. The same process was simultaneously completed in the freshwater buckets. A total of four trials were conducted, using newly randomised pair combinations of sticklebacks each time. When all trials were finished, the same procedure was as applied using the final density of 1000 larvae. Again, both freshwater and brackish water trials were run simultaneously.

3. Consumption rates as a function of stickleback group size

Both previous experiments involved using pairs of sticklebacks, however, sticklebacks are naturally found in larger shoal sizes. This experiment aimed to quantify any effects that group size may have on control efficiency. The percentage total of larvae consumed by ten different group sizes {1, 2, 4, 6, 8, 12, 16, 22, 28, 34} during a 15-minute feeding period was quantified in brackish water. Three buckets were set up in the tidal pool as short trial times made it impossible to run all group sizes simultaneously. Instead, four rounds of different group sizes per trial were required (Round 1 = groups 1, 34; Round 2 = groups 2, 4, 28; Round 3 = groups 6, 8, 22; Round 4; groups 12, 16). Each group was made up of randomly selected individuals and placed into a bucket of 50 larvae. After 15 minutes, sticklebacks were removed, remaining larvae were counted and the percentage of larvae consumed was calculated. This was repeated until all rounds in the trial were completed. The trial was replicated three more times, once a day over the following three days.

Procedure for counting larvae

As the first and third experiment required small samples of larvae per bucket (≤50 larvae), dip samples were taken from the bucket of collected larvae and placed in a white sampling tray. The required number of larvae were counted in the sampling tray before being added to a designated bucket. After each round or trial, sticklebacks were removed using a fine mesh aquarium net and placed in a plastic container of water. The container was checked for any larvae that may have been caught in the net with the sticklebacks. Buckets were left for several minutes to allow the sediments to settle before being lifted out slowly, keeping an upright position to allow most the water to drain out without larvae sticking to the sides of the mesh. The remaining water was quickly poured through the aquarium net and emptied in a white sampling tray, where the remaining larvae were counted and recorded.

For the second experiment that required multiple large larvae samples (>50 larvae), the larvae were counted by pouring a sample of water from one container into another, while counting larvae as they passed through the spout. This was found to be the most efficient way to count larger quantities of larvae as accurately as possible. The counted larvae were filtered through the aquarium net and placed into a designated experimental bucket. After each trial, the remaining larvae were counted in the same manner.

Abiotic parameters and Invertebrate Composition

Salinity (mS/cm), dissolved oxygen (% saturation) and temperature (°C) measurements were also taken from each treatment site. Six measurements of each parameter were taken in the permanent, tidal and isolated pool to calculate an average reading. Invertebrate composition of each pool type was also described to better understand the sub-habitats. Three invertebrate samples from each pool were collected by dabbing a hand-held net for 15 seconds along a random section of the pond perimeter. The invertebrate samples were placed into plastic bags with 70% alcohol for later identification. Due to COVID-19 restrictions, access to the lab was limited so the samples were identified at home, using a hand lens.

Statistical Analysis

Due to small number of replicates, Shapiro Wilk tests for normality and Levene's test for equal variances were performed for each dataset to confirm non-parametric distributions. Mann Whitney U tests were carried out to analyse the difference in the number of larvae remaining between the test and control buckets for each pool to confirm if sticklebacks were consuming mosquito larvae. This was followed up by Kruskal-Wallis tests to determine whether 1) there was a significant difference in larvae consumption across pool types and 2) to analyse the distribution of the control buckets across pool types. A Kruskal Wallis test with pairwise comparisons was also conducted to determine whether there were significant differences in the percentage of mosquitoes consumed between stickleback group sizes. All tests were conducted in SPSS.

RESULTS

Predator-prey interactions under three pool types

After 24 hours, the test buckets with sticklebacks had an average of 2, 2 and 1 larvae remaining in the permanent, tidal and isolated pool, respectively (Table 2). In the control buckets with no stickleback, the average number of remaining larvae were 16, 17 and 18 in the permanent, tidal and isolated pool, respectively (Table 2).

Table 2. Number of larvae left after 48 hours in test (stickleback) and control (no stickleback) buckets, and calculated averages of remaining larvae for each pool. Averages were rounded to the nearest whole number. Blank cells represent ineligible replicates due to disturbance of bad weather.

Pool Type	Replicate	TRIAL 1		TRIAL 2		TRIAL 3		Average	
		Test	Control	Test	Control	Test	Control	Test	Control
Permanent	1	6	14	2	14	1	14	2	16
	2	0	18	1	15	0	16		
	3	3	20	0	15	2	-		
Tidal	1	3	16	5	15	0	20	2	17
	2	0	15	5	20	2	-		
	3	0	20	0	16	0	-		
Isolated	1	0	16	2	20	3	20	1	18
	2	1	20	0	19	4	18		
	3	0	18	1	17	0	15		

A clear difference in the number of larvae left between all test and control buckets was identified (table 2, fig. 4), suggesting that sticklebacks are successful at attacking and consuming larvae in all three pool types. Differences in the number of larvae consumed by sticklebacks between each pool type were negligible, suggesting that stickleback feeding behaviour and consumption rates are not limited by different sub-habitats found in the marsh. Negligible differences were also observed in the number of larvae remaining across treatments (fig. 4), suggesting that mosquito larvae can survive in all three pool types. Low variability in test buckets, within and across each pool was evident. Despite the outlier in the permanent pool, the number of larvae left in each stickleback bucket were consistently less than 6 individuals (fig. 4). This low variability suggests that predator-prey interactions are consistent within and between pools.

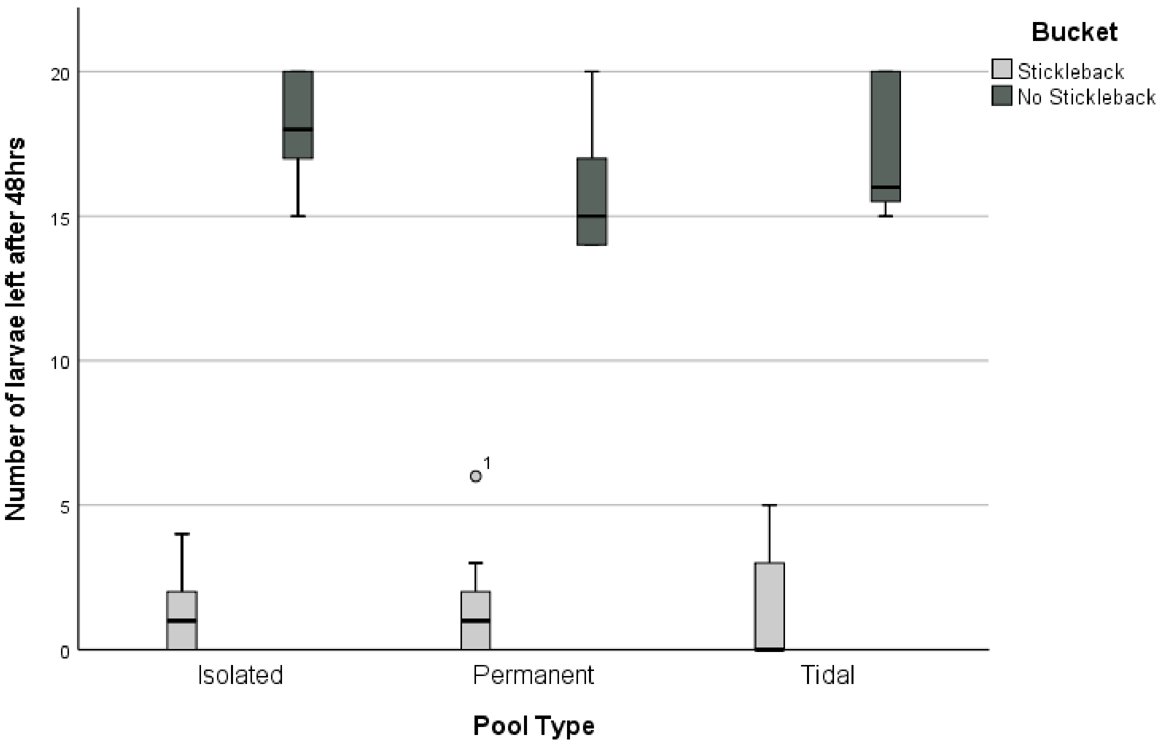

Fig. 4 Boxplot showing the distribution of remaining larvae in test and control buckets in each pool type.

These observations were strongly supported by Mann Whitney U tests and a Kruskal Wallis test. There was a significant difference ($P<0.05$) in the number of larvae remaining after 48 hours between the test buckets (n_1) and control buckets (n_2) ($U = 0$, $n_1 = 9$, $n_2 = 8$, $p <0.05$; $U = 1.0$, $n_1 = 9$ $n_2 = 7$, $p <0.05$; $U = 0$, $n_1 = n_2 = 9$, $p <0.05$, in the permanent, tidal and isolated pools, respectively). There was no significant difference in the number of larvae consumed between each pool type (X_2^2 = 0.231, N = 27, p = 0.891). There was also no significant difference in the number of larvae left in the control bucket (X_2^2 = 5.189, N = 24, p = 0.075)

Consumption rates as a function of prey density in freshwater vs brackish water

Table. 3. The number of mosquito larvae consumed by sticklebacks at different densities in freshwater and brackish water.

Density	Trial 1		Trial 2		Trial 3		Trial 4		Average ± SE	
	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish	Fresh	Brackish
50	42	43	39	46	49	46	48	48	45±2	46±1
100	85	87	82	98	91	93	96	95	89±3	93±2
200	179	188	188	177	191	177	195	197	188±3	185±5
300	252	258	273	268	288	271	289	269	276±9	267±3
400	296	353	322	322	379	358	358	286	339±18	330±17
600	350	351	488	427	475	442	403	373	429±32	398±22
1000	439	523	388	371	363	338	427	391	404±18	406±41

The relationship of stickleback consumption to increasing larval densities showed a Type II functional response, for both brackish and freshwater (fig 5), suggesting that sticklebacks are efficient at seeking out mosquito larvae at low densities. Sticklebacks also consumed a large number of individuals with an estimated maximum average consumption rate of 429 ± 32.29 larvae, per pair of sticklebacks, in 24 hours (table 3). Feeding rates began to slow down at a density of 400 larvae per bucket and plateau after 600 larvae per bucket (fig. 5). Overlapping error bars (fig. 5), indicate no difference in the functional response between freshwater and brackish water, suggesting that sticklebacks from the tidal creek are not sensitive to salinity changes and can attack and consume larvae at optimum levels throughout different sub-habitats in the marsh.

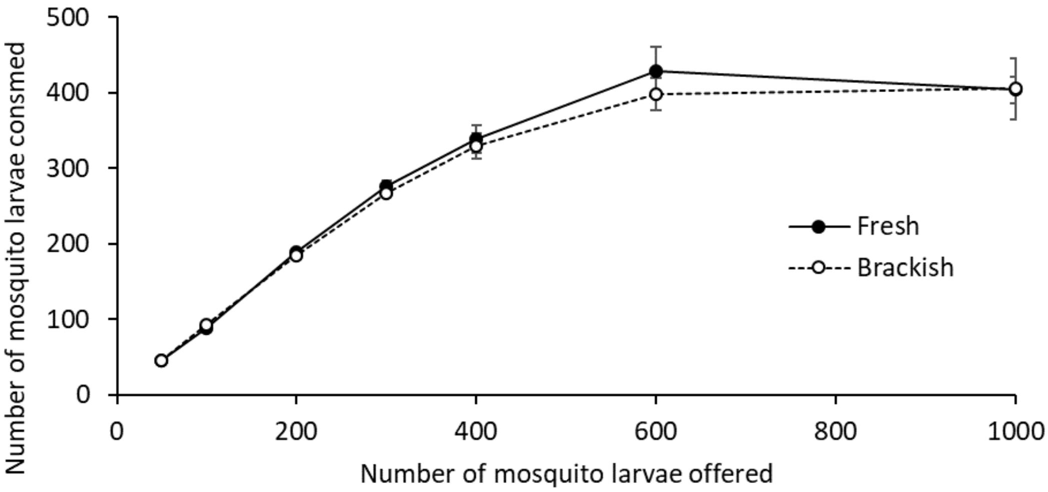

Fig. 5 Line graph showing the number of larvae consumed per two sticklebacks as a function of the total number of mosquito larvae offered (density) in fresh and brackish water

Consumption rates as a function of stickleback group size

Table 4. The % total of larvae consumed by lone and grouped sticklebacks when offered 50 larvae for 15 minutes.

Group size	Trial 1	Trial 2	Trial 3	Trial 4	average % consumed ± SE
1	10%	4%	6%	4%	6.0 ± 1.41
2	66%	36%	72%	60%	58.5 ± 7.89
4	60%	66%	56%	50%	58.0 ± 3.37
6	56%	48%	36%	74%	53.5 ± 7.97
8	58%	88%	80%	74%	75.0 ± 6.35
12	88%	70%	70%	58%	71.5 ± 6.18
16	72%	88%	90%	84%	83.5 ± 4.03
22	86%	100%	88%	100%	93.5 ± 3.77
28	76%	90%	90%	80%	84.0 ± 3.56
34	80%	98%	92%	86%	89.0 ± 3.87

When offered 50 larvae for 15 minutes, the percentage of total larvae consumed increased with group size (fig. 6), suggesting that sticklebacks are more efficient and faster at consuming larvae in larger group sizes. From fig. 5, the most efficient group size seems to be 22 individuals with 96% ± 3.77% of larvae consumed in 15 minutes, however, we cannot conclude this as the optimal size. A Kruskal Wallis and post hoc tests showed the percentage of larvae consumed in a group of 22 sticklebacks, is not significantly different from a minimum group size of 8 individuals (i.e., it is only significantly different from group with 1-6 individuals). However, small group sizes of 2-6 sticklebacks still consume a high percentage of larvae (e.g., two sticklebacks consumed an average of 58.5% of a total of 50 larvae which equates to 29 larvae in 15 minutes). As group sizes increase, the number of larvae consumed per capita decreases. This suggests that only a small number of sticklebacks are required to control smaller larvae populations while large groups of sticklebacks will successfully consume greater populations. Lone sticklebacks are not efficient at consuming larvae (6% ± 1.43 % of larvae consumed).

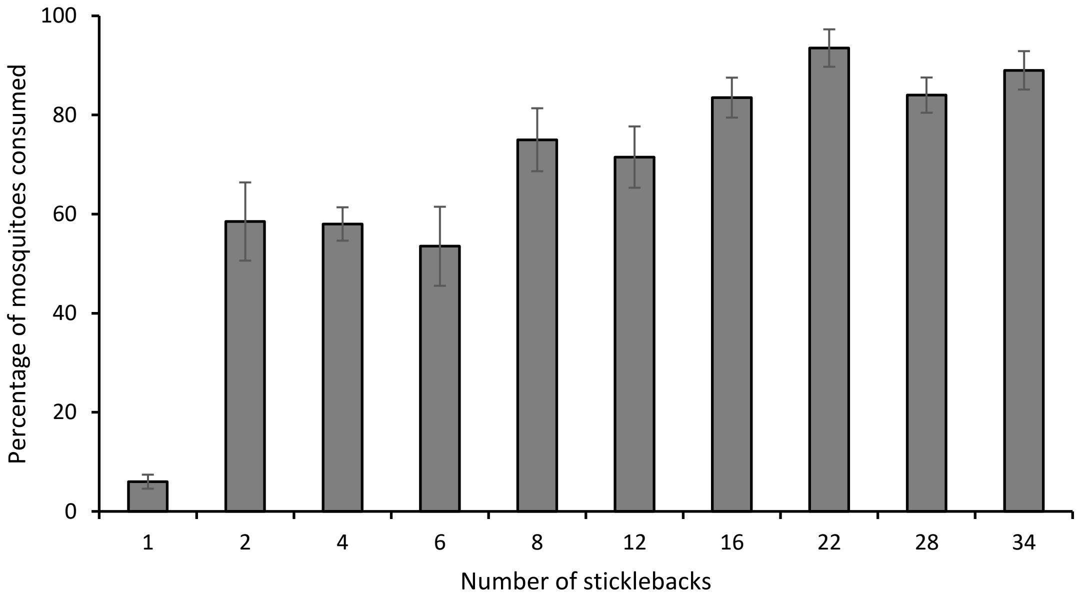

Fig. 6. Bar chart showing the average percentage of larvae consumed at 10 different group sizes.

Salinity, dissolved oxygen and temperature

Table 5. Average salinity (mS/cm), dissolved oxygen (% saturation) and temperature (°C) measurements taken in the permanent, tidal and isolated pool during the first experiment.

Pool Type	Salinity (mS/cm)	Dissolved O_2 (% saturation)	Temp (°C)
Permanent	10.9	49.4	11.3
Tidal	27.3	75.1	10.6
Isolated	13.4	43.4	11.1

The highest salinity and dissolved oxygen measurements were recorded in the tidal pool (27.3 mS/cm) and dissolved oxygen saturation (75 %), suggesting that it is subject to flushing from the tidal creek. There does not seem to be a major difference in the abiotic parameters between the isolated and permanent pool (table 5), in which larvae and sticklebacks were found respectively. This suggests that dissolved oxygen, salinity and temperature do not limit sticklebacks from colonising larval habitats. Similarly, it suggests that these parameters do no limit larval development in the permanent pool, where sticklebacks occur. It also shows that sticklebacks can colonise pools with low dissolved oxygen levels (49.4 % saturation).

Invertebrate composition

Distinct differences in invertebrate composition between study sites was evident as each pool showed different dominant families (table 5). The isolated pool was dominated by *Gammaridae* (69% composition), the tidal pool by *Corixidae* (81% composition) and the permanent pool by *Chironomidae* larvae (44% composition) (table 5). *Gammaridae* was the only family found in each pool, however, their percentage composition was highly varied between each pool type. *Gammaridae* was the most common in the isolated pool whereas only one individual was found in the tidal pool. A total of 10 families were identified.

Table 6. Invertebrate abundance and composition percentage (% of individuals from the total abundance) in the isolated, tidal and permanent pool.

Pool Type	Taxon	Name	Common name	Developmental stage	Abundance	%
Isolated						
	Family	*Gammaridae*	Gammarids/scuds	adult	132	69%
	Family	*Dytiscidae*	Diving beetle	larvae	19	10%
	Family	*Culicidae*	Mosquito	larvae	18	9%
				pupae	2	1%
	Family	*Gyrinidae*	Water beetle	adult	12	6%
	Class	*Collembola*	Springtail	adult	4	2%
	Family	*Helodidae*	Marsh beetle	larvae	2	1%
	Family	*Psychodidae*	Drain fly	larvae	1	1%
	Total abundance				190	
Tidal						
	Family	*Corixidae*	Water Boatmen	adult	25	81%
	Genus	*Sigara*				
	Family	*Palaemonidae*	Ditch shrimp	adult	3	10%
	Genus	*Palaemonetes*				
	Family	*Veliidae*	Riffle bugs	nymph	2	6%
	Family	*Gammaridae*	Gammarids/scuds	adult	1	3%
	Total abundance				31	
Permanent						
	Family	*Chironomidae*	Non-biting midges	larvae	18	44%
	Family	*Gammaridae*	Gammarids/scuds	adults	11	27%
	Family	*Dytiscidae*	Diving beetle	larvae	5	12%
	Class	*Collembola*	Springtail	adult	5	12%
	Family	*Gyrinidae*	Water beetle	adult	2	5%
	Total abundance				41	

DISCUSSION

Three-spined sticklebacks are efficient predators of *Culicinae* larvae and will consume larvae across different sub-habitats and salinities, including isolated pools where colonisation by sticklebacks does not occur. Mosquito larvae can also survive across different sub-habitats of the marsh; despite this, they only occur in distinct isolated pools. A minimum of two sticklebacks per group is required for effective control and larger group sizes will attack a higher percentage of larvae during a given time. Acknowledging pseudo-replicates and small sample sizes, it is evident that three-spined sticklebacks demonstrate strong potential for the biological control of mosquito larvae in Ballyvergan marsh. Furthermore, sticklebacks show consistency in the functional response between fresh and brackish water, and also a high tolerance to low dissolved O_2 levels and fluctuating salinities. This suggests that three-spined sticklebacks can provide biological control of mosquito larvae in freshwater, brackish or coastal waters that are subject to nuisance mosquito populations.

Although sticklebacks were not found in the tidal pool, it is likely that they do have access as suggested by the corresponding salinity of the tidal creek, measured during initial walkovers. Furthermore, ditch shrimp (*Palaemonetes*) were only present in the tidal pool and were also observed to be abundant in the creek. Sticklebacks do not occur in the isolated pool because it is not accessible from the tidal creek, as suggested by the negligible differences in the abiotic parameters and the absence of any connecting channels.

Since larvae only occur in habitats where sticklebacks cannot colonise, predator-prey interactions may be a driving force for gravid females when selecting a suitable habitat for oviposition. It has been found that *Culex* females use chemosensory cues to detect fish kairomones when selecting a suitable site for oviposition (Cohen and Silberbush, 2020). However, it is important to note that a series of other environmental factors are known to influence the suitability of larval habitats. Chemosensory cues are also used by gravid females to provide information on food availability, suitable conditions and competition (Afify and Galizia, 2015). Habitats chosen by saltmarsh mosquitoes are often characterised by areas with an abundance of dead, emergent or floating vegetation and not affected by tidal flushing (Medlock and Vaux, 2011). Some *Aedes* larval habitats are correlated with specific vegetation species (Gislason and Russell, 1997; Rowbottom et al., 2017; Service, 1968). These observations were also made in Ballyvergan marsh as mosquitoes only occurred in stagnant pools with lots of dry, emerging sedges and/or floating algae.

The combination of a voracious appetite represented by the functional response and high consumption rates in larger group sizes, implies that sticklebacks have the potential to control large numbers of larvae within a short period of time. The group size that shows maximum control efficiency could not be quantified in this study as resources and time were limited. The functional response curve that sticklebacks are effective at seeking out larvae even when larvae populations are low. However, the search efficiency at a low density under experimental conditions may not reflect true behaviour, as the white background of the bucket contrasts with the dark coloured larvae, potentially making it easier to spot prey.

It was proven that sticklebacks are effective predators of mosquito larvae in Ballyvergan marsh under controlled conditions. However, it is not guaranteed that these predator-prey interactions will have the same outcome under ‘natural’ conditions. Future studies involving connecting some of the isolated pools to the tidal creek by digging shallow trenches should be pursued to validate the findings in this study. It is also important to note, that when considering habitat modification such as OMWM, the effects on non-target species must be carefully taken into account (James-Pirri et al., 2011) and management must be applied and altered on a case to case basis (Wolfe, 1996). Although differences in invertebrate composition between each pool was evident, the importance and function of the invertebrate communities is unknown. Macroinvertebrates are reliable indicators of the ecological condition of saltmarshes (Weilhoefer, 2011; Wildsmith et al., 2011) and community functional composition provides information regarding food webs, trophic levels (Nordstrom et al., 2015) and nutrient cycling (Constable, 1999; Kraeuter, 1976). Therefore, an extensive study of invertebrate composition throughout the marsh may help to determine the impact of habitat modification on marsh function. Another future study could investigate the selectivity of sticklebacks towards mosquito larvae against other available prey in the marsh. If sticklebacks do not show a preference for mosquito larvae, their efficiency as a biological control predator is reduced. However, sticklebacks are potentially not the only predators of *Culicinae* larvae in Ballyvergan marsh. Ditch shrimp and diving beetles have been shown to be predatory invertebrates of mosquito larvae in UK marshes (Medlock and Snow, 2008). As ditch shrimp were observed to be highly abundant in the tidal creek, future research on the predator-prey interactions between ditch shrimp and mosquito larvae may potentially enhance control results. To ensure that the use of sticklebacks in Ballyvergan for mosquito control is sustainable, an investigation into the ecology of local sticklebacks is necessary. Understanding whether sticklebacks in Ballyvergan are resident in the permanent pool and tidal creek or migrate into nearby freshwater streams is essential to disclose their breeding sites. This may be of conservation concern as steady populations of sticklebacks would be required to guarantee prolonged control. Wider conservation interests need to be considered when considering larval control practices as marshes serve numerous valuable ecosystem functions and services.

Coastal marshes play a key role in combatting climate change issues as they provide protection from coastal erosion and from flooding during severe weather events (Möller et al., 2014). They also have potential for storing atmospheric CO_2 (Chmura, 2013). Due to their high ecological and economic value and in response to climate change, the creation and restoration of salt marshes are increasingly being practiced across north-west Europe (Boorman and Hazelden, 2017; Medlock and Vaux, 2011). Managed realignment is a soft engineering tool that describes the process in which reclaimed land is returned to an intertidal zone for the benefit of flood protection, restored biodiversity loss and mitigation of rising sea levels and coastal squeeze (Medlock and Vaux, 2015; Pétillon and Garbutt, 2008). While creating and restoring saltmarshes serve important functions in response to climate change, it may also promote suitable larval habitats and potentially bring an increased risk of nuisance and vector mosquitoes (Medlock and Vaux, 2015, 2013, 2011). Climatic conditions are changing in favour of nuisance and potentially disease bearing mosquitoes. Recent studies in the UK have found the common saltmarsh mosquito, *Aedes detritus,* to be a potential vector for Japanese encephalitis and West Nile virus (Blagrove et al., 2016; Mackenzie-Impoinvil et al., 2015). While there are no records of past endemic mosquito-borne human diseases in Ireland, a history of malarial fevers in Youghal during the late 19th/ early 20th century was reported by the landowner of Ballyvergan marsh (personal communication).

The direct and indirect consequences of climate change on mosquito populations are apparent, and opportunity for larval control of potential nuisance or vector species should not be overlooked. Ballyvergan marsh provides important regulatory abiotic services (Doran and T. O'Higgins, 2020) and is an important habitat for migratory birds (Cullen and Smiddy, 2008; Smiddy et al., 2007). There is an economic and social significance for the control of nuisance mosquitoes in Ballyvergan, as the marsh neighbours the touristic seaside town of Youghal and is located next to a mobile-home park. The marsh is also surrounded by grazing land and residential housing. An approved Greenway that is currently in the early stages of development, will provide better access to Ballyvergan marsh and attract a greater number of tourists to the marsh itself and the surrounding areas of Youghal. Integrative marsh management is a technique that aims to achieve the goals of both larval control and marsh restoration (Rochlin et al., 2012), and may be of interest in Ballyvergan due to its far-reaching ecosystem services. Targeting mosquito larvae populations through the predation of three-spined sticklebacks while maintaining the marsh's ecological and ornithological value should be considered for future management plans.

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